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Award Review

Chemical Ecology of Insect-Plant Interactions: Ecological Significance of Plant Secondary Metabolites

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Abstract

Plants produce a diverse array of secondary metabolites as chemical barriers against herbivores. Many phytophagous insects are highly adapted to these allelochemicals and use such unique substances as the specific host-finding cues, defensive substances of their own, and even as sex pheromones or their precursors by selectively sensing, incorporating and/or processing these phytochemicals. Insects also serve as pollinators often effectively guided by specific floral fragrances. This review demonstrates the ecological significance of such plant secondary metabolites in the highly diverse interactions between insects and plants.

Key words: insect-plant interaction; host-finding cue, defensive substance; pheromone; synomone

Insects are the most diverse group of animals on the earth, representing more than half of all known living organisms. While most of insects rely on plants' primary metabolites (e.g. carbohydrates, lipids and proteins), plants have evolved a high diversity of secondary metabolites (e.g. alkaloids, terpenoids, phenolics) to cope with heavy herbivory. Insects are continuously challenged with counter mechanisms to detoxify or circumvent plants' defense systems. On the other hand, insects and plants have coevolved mutualistic relationships through pollination. In such a complex ecological network, insects have developed highly sensitive and specific chemical sensors and biochemical processes to detect allelochemicals - to find host plants and nectar sources, to avoid noxious plants and to escape from predators, in addition to their intraspecific recognition via pheromonal communications.

This review focuses on the chemistry and ecological significance of plant secondary metabolites involved in insect life histories and emphasizing particularly the following 5 subjects that are often closely linked to each other – partly summarizing my 45 years of research in insect chemical ecology.

I. Plant metabolites as host-finding cues

II. Plant metabolites as chemical barriers

III. Plant allelochemicals for defense

IV. Plant metabolites for sexual communication

V. Floral volatiles in mutualistic associations

I. Plant metabolites as host-finding cues

Most phytophagous insects feed on a limited number of plant species that often belong to a single plant family. The choice of host plants is determined both at the egg-laying and larval-feeding stages.[1)] Insects' host recognition involves multiple sensory modalities, including visual, olfactory, gustatory and tactile cues. My attention has been focused on the phytochemical factors controlling egg-laying and larval feeding behavior in swallowtail butterflies and sap-sucking behavior in aphids. These insects respond to specific chemical ingredients in the host plants.

1. Oviposition stimulants of swallowtail butterflies

"How can a citrus swallowtail butterfly sharply distinguish her host plants such as citrus and zanthoxylum-pepper trees, from many other neighboring plants?" was a question in my mind since childhood. The selection of an oviposition site by an adult female is crucial to the survival of her offspring, because the larvae are destined to eat only selected plant species. Thus, the mother butterflies must lay their eggs with great precision on the host plants. Females of swallowtails detect specific chemicals in the host plants by vigorously drumming upon the leaf surface with their forelegs. Toothbrush-like chemosensilla were found on the foretarsi of the female butterflies.[2)] When a piece of filter paper impregnated with a host plant extract was brought in contact with the tarsal sensilla, she immediately deposited one or more eggs on the substrate.

The complex chemical profiles of oviposition stimulant systems have been

revealed step by step in several papilionid species mainly by Keiichi Honda (Hiroshima Univ.), Paul Feeny's group (Cornell Univ.) and our group (Nishida, Kyoto Univ.), and providing a fairly systematic model for understanding the chemical basis and evolutionary process of host selection in phytophagous insects.

Rutaceae-feeders: Oviposition stimulants of a citrus swallowtail butterfly, *Papilio xuthus* in *Citrus unshiu* leaves were found to consist of multiple components, which included flavonoids (hesperidin (**1**), narirutin (**2**), rutin (**3**), vicenin-2 (**4**)), a nucleoside (adenosine (**5**)), alkaloids ((–)-synephrine (**6**), 5-hydroxy-*N*ω-methyltryptamine (**7**), bufotenine (**8**)), a cyclitol ((+)-*chiro*-inositol (**9**)) and an amino acid derivative ((–)-stachydrine (**10**)) (Fig. 1).[3-5] None of the individual components elicited oviposition responses alone. The specific activity was provoked only when these compounds were applied as a mixture, indicating the importance of a synergistic effect of multiple components in host recognition. Honda[6] elucidated the oviposition stimulants of another citrus-feeding swallowtail, *P. protenor*, which also shares the same host, *C. unshiu*. Among the 7 stimulant components, 3 components (**1**, **6** and **10**) were shared by *P. xuthus* and *P. protenor*.[7] More than 70 swallowtail species in the tribe Papilionini are known to feed on the citrus family (Rutaceae). The oviposition stimulants of *P. macilentus* and *P. bianor*, which share a common rutaceous host, *Orixa japonica*, were characterized as cnidioside A (**11**) (*P. macilentus*, unpublished result) and a mixture of a hydroxylated γ-lactone (**12**) and a hydroxycinnamoyl ester (**13**) (*P. bianor*), respectively, demonstrating the distinct difference in the host finding cues between these two sympatric species.[8,9]

Apiaceae-feeders: Females of an Apiaceae-feeding common yellow swallowtail, *P. machaon hippocrates*, "mistakenly" lay eggs on plants belonging to Rutaceae family, such as *Poncirus trifoliata* and *Zanthoxylum piperitum*, common hosts of *P. xuthus* and *P. protenor*. The same reaction can be induced by stimulating a *P. machaon* females with extracts of these plants, although the response is much weaker than that induced by an extract from a carrot leaf, one of its major host, suggesting some common

components involved as oviposition stimulants between Apiacea and Rutaceae.[2] The oviposition stimulants of another Apiaceae-feeder, *P. polyxenes*, was identified as a blend of a malonylated flavone glycoside (**14**), tyramine (**15**) and chlorogenic acid (**16**).[10,11] The synergistic effects of combinations of flavonoids, hydroxycinnamates and phenetylamine derivatives appeared to be a common feature of oviposition stimulant system among these two closely related butterfly species, even though the host plant taxa (Apiaceae and Rutaceae) are remote to each other. Such an underlying phytochemcial similarity might have provided a route to colonization on novel hosts among the papilionid butterflies.[12,13]

Aristolochiaceae-feeders: Most of the swallowtail butterflies in the tribe Troidini and Zerynthiini feed exclusively on the Aristolochiaceae. The oviposition stimulants of the troidine species *Atrophaneura alcinous* were isolated from the aerial parts of a pipevine, *Aristolochia debilis*, and identified as a mixture of aristolochic acids (AAs) (AA-I, **17**) and sequoyitol (**18**).[14] AAs bear a nitro group on the phenanthrene ring, unique alkaloidal metabolites contained exclusively in the Aristolochiaceae, and thus would serve as an excellent cue. However, AAs exhibited very low activity alone. This represents an importance of synergism between **17** and **18**. In addition, 3-hydroxy-4-methoxycinnamoylmalic acid (**19**), closely related to hydroxycinnamoyl esters (**13** and **16**) utilized as a chemical cue by several Rutaceae-feeders, was involved as an additional factor in *A. alcinous* (unpublished results). Further, pinitol (**20**), a hybrid compound between **9** and **18** was characterized as an oviposition stimulant for another troidine, *Battus philenor* together with **17**.[15] One of the oviposition stimulants of a primitive zerynthiine swallowtail, *Luehdorfia japonica*, was isolated from the leaves of *Heterotropa aspera* and identified as a new flavonol triglycoside (**21**).[16] The tribe Zerynthiini, to which *L. japonica* belongs, is considered to represent remnants of the stock from which the rest of the Papilionidae evolved.[17] Utilization of flavonoids as the oviposition stimulant components by both the most advanced genus, *Papilio*, and one of the most primitive genera, *Luehdorfia*, suggests the underlying conservative

nature in host recognition within the family Papilionidae. Flavonoids seem certainly to provide a phytochemical diversity and specificity together with other stimulant ingredients as the host finding cues for these phytophagous insects.

2. Larval phagostimulants of swallowtail butterflies

The cabbage white butterflies, *Pieris rapae* and *P. brassicae* (Pieridae), recognize host crucifers by glucosinolates both during oviposition and larval feeding.[18-20] Likewise, larvae of an Aristolochiaceae-feeding swallowtail, *A. alcinous*, were found to be stimulated by the host specific secondary metabolites **17** together with unknown cofactors.[14,21] These facts suggested a congruent sensory mechanism between the tarsal chemoreceptors of adults and the gustatory chemoreceptors of larvae towards host-specific plant allelochemicals. However, the nature of such chemosensory processes at both larval and adult stages is not well understood.

Larval feeding stimulants for a Rutaceae-feeding swallowtail, *P. xuthus*, were examined in *Citrus unshiu* leaves in comparison with the oviposition stimulants described above, using thin paper strips as a substrate. Stimulation of feeding on the extracts was found to require a mixture of chemicals including sugars (D-glucose, D-fructose, and D-sucrose), stachydrine, a cyclic peptide (citrusin I (**22**)), a polymethoxyflavone (isosinensetin (**23**)) and lipids (i.e. four 1-monoacylglycerols and a glycoglyceride (**24**)) (Fig. 1).[22] The larvae readily consumed the test strips treated with a mixture of all the 11 compounds, while very few larvae consumed the strip when each compound were tested alone, indicating that host recognition by *P. xuthus* larvae is mediated by a specific combination of both primary and secondary metabolites. This result is in contrast to the 10 oviposition stimulant components of *P. xuthus* contained in the same host species (*C. unshiu*) in which only one compound, stachydrine (**10**), was an ingredient in common. While the larval feeding-stimulant mixture is dominated by nutrients and other compounds of general significance for primary metabolism, the component oviposition stimulants are exclusively secondary substances that have fairly

restricted distributions in plants. Since the initial host choice for the larvae is made by the ovipositing female, unique secondary metabolites may be less important cues for larval feeding than are compounds useful for indicating food and microhabitat quality once settled on the host plant. Likewise, our recent study revealed that the larvae of a primitive Aristolochiaceae-feeding swallowtail butterfly, *Sericinus montela* (tribe Zerynthiini), were stimulated to feed by a mixture of secondary substances specific to the host *A. debilis* (**17** and **18**) plus a series of ubiquitous sugars (glucose, fructose, sucrose) and a lipid (**24**). The complex blend of these primary compounds was identical to the key larval feeding stimulants of *P. xuthus* (unpublished results). This result further substantiates the common nature of both primary and secondary plant metabolites serving as gustatory cues to which oligophagous lepidopteran larvae are tuned.

Initially, the complexities of host recognition and feeding stimulation were demonstrated in some lepidopteran larvae: some volatile essential oil components were found to act as olfactory cues in determining the choice of host plants prior to contact with the leaves by larvae of the Apiaceae-feeding swallowtail species, *P. polyxenes*.[23] In the silkworm moth, *Bombyx mori* (Bombycidae), larval feeding behavior is controlled by three distinct factors: attractants (e.g. citral, linalool, 3-hexenol), biting factors (e.g., isoquercitrin, β-sitosterol), and swallowing factor (cellulose) in addition to cofactors (e.g. sucrose, *myo*-inositol, inorganic phosphate)[24]; a recent study has revealed an olfactory receptor protein responsible for a key mulberry leaf volatile, jasmone.[25] Elucidation of olfactory and gustatory chemical cues and their chemosensory mechanisms of host recognition both at egg-laying and larval feeding will contribute to a greater understanding of the evolution of host selection in Lepidoptera.

3. Probing stimulants in aphids

Aphids (Aphididae) are mostly sap feeders that gregariously suck plant nutrients from the phloem of still developing buds and leaves. Although many agricultural pest

species are polyphagous, infesting a wide rage of crops and often alternating seasonally between hosts, many other species are oligophagous within a limited number of host species belonging to a single family. Van Emden (1972)[26] declared that “aphids are phytochemists”, i.e. their life histories are greatly influenced by plant chemistry. The host ranges of the Aphididae are far more diverse than those of butterflies and include ferns, gymnosperms and angiosperms, yet aphids exhibit some similarities in host selectivity to each other among some oligophagous species. Thus, my studies on aphids were conducted in parallel to those on butterflies to clarify the chemical bases of host specificity among several aphid species.

The process of finding food sources in aphids involves the following sequential steps: (1) orientation to a plant, (2) external examination, (3) probing through the plant tissues, (4) tapping the phloem, (5) ingestion.[27] Specific chemical cues seem to be associated within each step during the assessment of suitable hosts.

My research group has elucidated chemical factors that regulate step (3) to (5) in an oligophagous bean aphid, *Megoura crassicauda*, which feeds selectively on plants in the genus *Vicia* (Fabaceae) and is known as a pest of broad bean, *V. faba*. Two specific probing stimulants were isolated from one of its favorite hosts, narrowleaf vetch, *V. angustifolia*, and were characterized as acylated flavonol diglycosides (**25** and its glucosidic isomer).[28] These compounds strongly induced formation of a proteinous stylet sheath on a parafilm membrane when applied as a solution in distilled water, suggesting that they act as a factor in navigating the stylet towards the phloem in step (3). In contrast, their corresponding deacyl analogs, present abundantly in the host tissues, were suggested to serve as a negative stimulus to allow the aphid to refrain from sucking during tissue penetration before tapping the phloem, although the distribution of these analogs in the plant tissues remains unknown. Chemical factors in the host plant extract involved in the feeding steps (4) and (5), were represented by sucrose and amino acids, because the aphids positively imbibed an artificial diet composed of primary nutrients mimicking a phloem sap in addition to the probing stimulants and

produced a large quantity of honeydews.[29] Thus, the feeding behavior of *M. crassicauda* was shown to be controlled by multiple chemical stimuli composed of primary and secondary plant metabolites mediating the process of the settlement on its hosts.

A dihydrochalcone, phlorizin, is known as a host-specific chemical cues for an apple aphid *Aphis pomi*.[30] We have also identified the probing stimulants of a cowpea aphid, *Aphis craccivora* (host broad bean, *Vicia faba*), rose aphid, *Sitobion ibarae* (host Japanese rose, *Rosa multiflora*) and an elder aphid, *Acyrthosiphon magnoliae* (winter host elder, *Sambucus racemota*) to be flavonol glycosides present in the corresponding hosts (unpublished results). These results provide additional examples of utilization of flavonoids as kairomonal cues by aphids.

II. Plant metabolites as chemical barriers

Besides insecticidal and other noxious plant allelochemicals, there is a diverse array of secondary metabolites to disrupt processes such as insect host-finding behavior and endocrinological systems. This section illustrates the ecological and physiological roles of some of these phytochemicals as potential chemical modulators of phytophagous insects' activities.

1. Oviposition and feeding deterrents in butterflies

Although the citrus swallowtail, *P. xuthus*, feeds on various rutaceous species, both the female butterflies and larvae avoid a rutaceous plant, *Orixa japonica*, due to potent deterrent chemicals in the plant. The most abundant flavonoid triglycoside (**26**) was characterized as one of the major oviposition deterrents from the leaves (Fig. 2).[31] This compound is a xylosyl derivative of rutin (**3**), a positive ovipositional stimulant for the butterfly (Fig. 1), and thus may possibly disrupt the oviposition stimulant activity due to its structural resemblance, competing for the same chemoreceptor neurons on the foretarsi. Further, two hydroxybenzoic acid derivatives were characterized as potent

deterrents of both oviposition and larval feeding in *P. xuthus*: a glycosylated hydroxybenzoic acid (**27**) and disyringoyl glucaric acid (**28**).[32] Simultaneous occurrence of these compounds in *O. japonica* appears to provide an effective chemical barrier against *P. xuthus*. This suggests a congruent chemosensory mechanism of allomonal chemicals acting on both female tarsal chemoreceptors and larval maxillary taste receptors, which can be contrasted to the case of oviposition stimulants/larval feeding stimulants in *P. xuthus* as discussed above (**I**-1 and **I**-2).

2. Probing deterrent in aphids

As mentioned in **I**-3, the bean aphid, *M. crassicauda*, feeds selectively on plants of the genus *Vicia*. However, it never infests the tiny vetch, *V. hirsuta*, even though the plant often forms a mixed community with *V. angustifolia*, one of the most favorite hosts of the aphid. A specific probing deterrent was isolated from *V. hirsuta* by monitoring the inhibitory effect, and its structure was elucidated as a hemiterpene glucoside (**29**).[33] Because the stylet insertion process is a crucial step for the aphid's settlement on a plant, the deterrent glycoside seems to act as an effective chemical barrier for *V. hirsuta*.

3. Insect growth regulators

Plants even manufacture exact insect hormone molecules such as 20-hydroecdysone and juvenile hormone (JH) III (**30**) in massive quantities.[34,35] During my collaborative investigations of hormonal substances with Professor William S. Bowers at Cornell University, two novel compounds, juvocimenes I and II (**31**), with an extraordinarily high JH activity were isolated from an essential oil of sweet basil, *Ocimum basilicum* (Labiatae).[36] Juvocimenes possess a fused structure between (*E*)-β-ocimene (monoterpene) and (*E*)-anethole (phenylpropanoid).[37] Juvocimene II showed about 3000 times as much activity as that of JH I in the milkweed bug,

Oncopeltus fasciatus in a topical application. Similarly, a compound with a significant JH activity was isolated from the plant, *Macropiper excelsum* (Piperaceae), and was determined as 1-(3,4-methylenedioxyphenyl-(*E*)-3-decene (**32**) (named juvadecene).[38)]

It is of great interest if plants have developed specific biosynthetic pathways in response to intensive herbivory by modifying existing molecular units to make a novel arsenal as mentioned above: oviposition deterrent flavonoid triglycosides (**26**) with rutin (**3**) + xylose; juvocimenes (**31**) with monoterpene + phenylpropanoid; juvadecene (**32**) by extension of side chain of a phenylpropanoid commonly present in Piperaceae.

III. Sequestration of plant allelochemicals for defense

A number of phytophagous insects seem to have been selected to sequester plant allelochemicals in their body tissues and/or integuments.[39,40)] Thus, such insects can obtain a potent defense mechanism without manufacturing noxious chemicals of their own. Sequestered phytochemicals include highly toxic alkaloids, terpenoids and phenolics, which normally function in plants to repel or poison herbivores. This section presents examples of a variety of sequestrates and their defensive modes of action against potential predators.

1. Recycling of larval host chemicals for defense

Pipevine swallowtails - aristolochic acids: A number of aposematic swallowtail species belonging to both tribe Troidini and Zerynthiini feed on the Aristolochiaceae and sequester a series of toxic AAs from their host plants for defense.[40-42)] Larvae of *A. alcinous* selectively incorporate AAs from their host plant, *Aristolochia debilis*, into their body tissues. A large titer was found in eversible defensive glands (osmeteria) as a concentrated solution in glycerol.[21)] AAs act as a feeding deterrent for bird predators. AAs are present in pupal and adult tissues as well as in eggshells and yolk, which suggests there is an effective protection of every life stages from potential enemies by

depending solely upon the host pipevine. Since AAs act as host-finding cues both by the adult female butterflies (**I**-1) and larvae and (**I**-2), an assessment of the host-plant quality both at the oviposition and larval feeding stages could guarantee subsequent protection.[14] Ecological adaptation of *A. alcinous* thus seems to be strongly associated with sensory mechanisms specifically developed for perception of AAs.

Danaine butterflies - pyrrolizidine alkaloids: Most danaine butterflies acquire pyrrorizidine alkaloids (PAs) during their adult stage by foraging for non-host plants rich in PAs. However, a giant danaine butterfly, *Idea leuconoe* (Nymphalidae), in Okinawa feeds on *Parsonsia laevigata* (Apocynaceae) and sequesters a series of pyrrolizidine alkaloids including new macrocyclic alkaloids, ideamines B (**33**) and C as *N*-oxide forms, directly from its host plant (Fig. 3).[43,44] The alkaloids are considered to function as defensive substances against predators, and are used as pheromone precursor by males during courtship (see section **IV**). Most other danaine species acquire PAs not from the hosts but as adults by foraging from PA-containing plants (see **III**-2). It has been suggested the host *Parsonsia* is one of the ancestral food plants of the Danainae, and the insects retained the ability to acquire PAs through pharmacophagy even after they shifted to other plants.[45] This primitive butterfly may provide a clue for understanding the evolution of pharmacophagous behavior in the subfamily Danainae.

Apollo butterflies - cyanoglucoside: It was initially found that a number of day-flying *Abraxas* moth species (e.g. *A. miranda*, *A. latifasciata*, *A. glossulariata* etc., Geometridae) feeding on leaves of *Euonymus* spp. (Celastraceae) during the larval stage store a large quantity of a bitter-tasting cyanoglucoside, sarmentosin (**34**), in the adult body tissues from the larval hosts.[41,46] Sarmentosin itself is not cyanogenic, but its epoxide spontaneously liberates HCN (Nahrstedt, 1982)[47]. During discussion with Dr. Miriam Rothschild, a possible involvement of sarmentosin with Apollo butterflies (*Parnassius* spp., Papilionidae) was suggested, because they feed on *Sedum*, which was reported to contain sarmentosin. As predicted, sarmentosin was detected in a substantial quantity in adults of *P. apollo* in Europe and in *P. phoebus* in the Rocky Mountains in

the USA.[42,48] Thus, the secret of their aposematic life style both as larvae and adults can be explained by sermentosin, although any potential predators and the actual defensive roles of the compound remain to be clarified.

Ericaceae-feeding moth - grayanoid diterpenes: Grayanotoxins (GTs, grayanoids) are highly oxygenated diterpenes with 5 and 7 membered rings, unique in ericaceous plants. These compounds exhibit potent neurotoxicity in mammals by binding to specific sodium ion channels in cell membranes, and thus herbivorous animals avoid the plant .[49] However, larvae of the leopard moth, *Arichanna gaschkevitchii* (Geometridae), selectively eat with impunity the young leaves of an ericaceous plant, *Pieris japonica*, even though it contains high concentrations of GTs. Several GTs, including two new analogs, arichannatoxins I (**35**) and II, were identified from the body tissues of adult moths in a concentration as high as 300 μg/moth, which was sufficient to deter predatory house lizards.[41,50] The adult moth exhibits an aposematic color pattern with orange-yellow hind-wings and it flies during the day like a butterfly. A related species with a similar wing pattern, *A. melanaria*, oligophagous on several ericaceous plants often lacking GTs, sequesters much lower amounts of GTs when fed on *P. japonica* (less than 60 μg/moth). This represents a case of Müllerian mimicry in the same genus, sharing the same host, with different loads of the defense substances.[40]

Rubiaceae-feeding aphid - Iridoid glucoside: A ruby-red colored aphid, *Acyrthosiphon nipponicus* (Aphididae), monophagously feeds on *Paederia scandens* (Rubiaceae). The aphid is seldom attacked by the ladybird beetle, *Harmonia axyridis* (Coccinellidae). A potent deterrent against the beetle was isolated from the aphid and identified as paederoside (**36**), an iridoid glycoside containing a sulfur atom in the molecule, unique in the aphid's host, *P. scandens*.[51] The aphids secrete a mixture of **36** and lipids from the cornicles (aphids' defensive glands), and quickly smear the fluid onto a predator's mouthpart. Once an adult of *H. axryridis* bites into the aphid, it immediately releases the aphid, regurgitates, and quickly flees from the aphid colony. This exemplifies a typical case of usurpation of plant's defensive substance by a

phytophagous insect effectively to repel its major predator in a tritrophic interaction. The effect of the aposematic red color of the aphid against the ladybirds is not known. Kamo et al.[52] recently reported that the black-colored cowpea aphid, *Aphis craccivora*, that infests the black locust, *Robinia pseudoacacia* (Fabaceae), showed toxicity to *H. axyridis* due to cyanamide (NH_2-CN), whereas the same aphid that infests the common vetch, *V. angustifolia* (free from the toxin), is suitable prey for *H. axyridis* larvae, further exemplifying this multitrophic adaptation mechanism in aphid-host plant interactions (cf. **I**-3).

2. Pharmacophagy - sequestration of non-host plant chemicals

"Plants are not only a 'grocery store' for feeding, but can also be a 'pharmacy'", this observation by Boppré (1984)[53] being the basis for his metaphorical term, "pharmacophagy". If an animal acquires plant substances not for primary metabolism but for specific purpose other than nutrition or host recognition, it is defined as pharmacophagous. Adults of many danaine butterflies and some arctiid moths gather PAs pharmacophagously from specific plants rich in the alkaloids instead of acquiring them from their larval hosts (cf. *Idea leuconoe*, **III**-1)[42]. Several other pharmacophagous associations have been found in Diptera, Hymenoptera and Coleoptera, as described bellow[54].

Turnip sawfly - *neo*-clerodane diterpenoids: Adults of the turnip sawfly, *Athalia rosae ruficornis* (Tenthredinidae), visit a plant, *Clerodendron trichotomum* (Verbenaceae), and feed voraciously on glandular trichomes on the leaf surface. A series of bitter-tasting *neo*-clerodane diterpenoids (e.g. clerodendrins A, B, D (**37**) were characterized as the potent phagostimulants for the sawfly (Fig. 3). [54,55] The insect subsequently sequesters some of the analogs and likely becomes extremely bitter to predators on its body surface. The major sequestrate **37** deterred feeding by sparrows and tree lizards.[54,56]

Leaf beetle - cucurbitacin triterpenes: Polyphagous leaf beetles, *Diabrotica*

speciosa and *Cerotoma arcuata* (Chrysomelidae) are destructive pests of various crops in Brazil. They are strongly arrested by root components from the cucurbit plant, *Ceratosanthes hilariana* (a non-host for larvae). Although the major phagostimulants were identified as cucurbitacins B and D, the adults selectively accumulated a relatively more stable minor component, 23,24-dihydrocucurbitacin D (**38**), gaining bitterness effective against a bird predator.[54,57] Similarly, four Asian Cucurbitaceae-feeding specialists in the genus *Aulacophora* were found to sequester the same compound directly from the larval/adult host cucurbits.[58] The strong affinity to cucurbitacins, selective sequestration of the analogs and consequent protection from predators suggested an ecological adaptation mechanism developed in common among these two geographically isolated subtribes in the Luperini - both of the New and Old Worlds.[58]

Oriental fruit fly - methyl eugenol: The oriental fruit fly, *Bactrocera dorsalis* (Tephritidae) is a serious agricultural pest, infesting most succulent fruit species in the tropics and subtropics. Methyl eugenol (ME, **44**) is known as an extremely potent attractant for *B. dorsalis* males (Fig. 4), and the compound was successfully used as a mass-trapping agent in eradication programs such as in Okinawa.[59] The males attracted to the chemical source voraciously feed on the intact chemical and sequester its metabolites, 2-allyl-4,5-dimethoxyphenol (**45**) and (*E*)-coniferyl alcohol (**46**), selectively in the rectal glands known to be a sex pheromone reservoir.[60] The total amount of the metabolites accumulated in the rectal glands was often as high as 100 μg/male, sufficient to deter feeding by predators, such as sparrows, lizards and spiders.[54,61] Because **44** is distributed widely in plants, males can acquire the compound pharmacophagously by foraging during adulthood.[62] Many host fruits of *B. dorsalis* contain **44** as a minor component. However, the larvae fed on an artificial diet containing **44** did not carry over the compounds to the adults.[63] Pheromonal roles of ME-metabolites are discussed in Section **IV**, and synomonal associations are discussed in Section **V**.

IV. Plant metabolites for sexual communication

Insects produce a diverse assemblage of sex pheromones to attract the opposite sex of the same species. These compounds are strictly species-specific with unique chemical structures and/or combinations of multiple components manufactured by specific biosynthetic pathways. However, in some instances, insects employ specific plant secondary metabolites to attract and excite the partners during courtship.

1. Plant-derived sex pheromones

Giant danaine butterfly - pyrrolizidine alkaloids: Males of the danaine butterfly, *Idea leuconoe*, display 'hairpencils', a pair of brush-like glandular organs, to cause the female to become quiescent during courtship. The hairpencil volatiles are composed of a complex mixture, including danaidone (**40**) and viridifloric β-1actone (**41**) (Fig. 4).[64,65] Virgin females were visually attracted to and olfactorily arrested by an artificial butterfly model scented with a blend of the two PA fragments **40** and **41**. These two compounds appeared to be fragments of a pyrrolizidine alkaloid such as lycopsamine **39** originated from the host, *P. laevigata*, and sequestered in the body tissues as *N*-oxide forms for defense (see Section **III**-1). The female seems to verify a male's ability to provide protection via the quality of pheromone volatiles emitted from hairpencils. This strong allelochemical linkage by this primitive butterfly suggests that a secondary colonization of danaine species to PA-free plants such as Asclepiadaceae might have taken place with retention of the PA-mediated allomonal and pheromonal systems via pharmacophagous acquisition of the precursors from non-host PA plants.[42]

Oriental fruit moth - herbal essence: Males of the oriental fruit moth, *Grapholita molesta*, emit a herbal scent, such as methyl epijasmonate (**42**) and ethyl (*E*)-cinnamate (**43**), from hairpencils to attract conspecific females after himself attracted to the female's vicinity by the female pheromone (e.g. (*Z*)-8-dodecenyl acetate).[66] Ester **42**, known as the key ingredients in high-grade perfumes originally extracted from jasmine flowers, is a plant hormone that regulates various physiological processes. These herbal

essences are presumably derived from their larval host fruits, although the origin of these compounds remains to be clarified.[67)]

Oriental fruit fly - phenylpropanoids: The strong attractiveness of *B. dorsalis* males to phenylpropanoid **44** (ME) is a unique characteristic to obtain a defensive measure as discussed above (see Section **III**). Males fed with **44** significantly had their performance during courtship enhanced, and they competed for virgin females significantly better than ME-deprived males.[68-70)] The pharmacophagously acquired ME-metabolites **45** and **46** were shown to arrest females, inducing their acceptance posture as illustrated in Fig. 4. Males of several other closely related *Bactrocera* species (*B. carambolae*, *B. zonata*, *B. correcta*) fed on ME selectively converted it into other unique subsets of ME-metabolites, including (*Z*)-coniferyl alcohol and (*Z*)-3,4-dimethoxycinnamyl alcohol, and stored these in the rectal gland.[71,72)] A series of sesquiterpene hydrocarbons such as β-caryophyllene (**47**) was identified from the rectal gland of wild *B. correcta* males.[73)] These pharmacophagously acquired rectal sequestrates (both phenylpropanoids and sesquiterpenes) were suggested to function possibly as a unique sex pheromone to attract conspecific females of this highly diverged species radiating from central Southeast Asia. Compound **47** was found to be a more attractive male lure than **44** in *B. correcta*, and **47** thus serves as a highly selective monitoring agent not attractive to other sympatric pest species in Thailand (unpublished results).

In these instances, the male pheromone systems are suggested to have advanced within the context of sexual selection – the female's preference for the specific components linked to fitness-related defensive compounds. These plant-originated male pheromones may have evolved possibly through a sensory exploitation process to appeal to the female's chemoreceptors, and as a consequence females recognize males that are coincidentally highly endowed with a defensive armament (Nishida, 2002).[42)] These male-initiated pheromone systems can be contrasted to the case of the turnip sawfly, *A. rosae ruficornis* (see Section **III**-2), wherein females pharmacophagously

acquire clerodendrins (e.g. **37**) from *C. trichotomum* and males select females loaded with the bitter-tasting diterpenoids.[74] Sexual selection in these cases appeared to be tightly linked with defensive fitness properties arising from natural selection via plant secondary metabolites.

2. Plant signals for rendezvous cues

Observation of patrolling males of the citrus swallowtail butterflies, *P. xuthus* and *P. protenor*, searching for females along the edge of citrus bushes suggests an involvement of some chemical cues from the hosts (personal observations). However, a possible role of the host odor in this precopulatory step has rarely been investigated.[75]

Mediterranean fruit fly - α-copaene: A sesquiterpene hydrocarbon, α-copaene (**48**), initially found in *Angelica* oil as a potent attractant for males of the Mediterranean fruit flies, *Ceratitis capitata* (Tephritidae), is also found as a minor leaf volatiles of various plant species, including its major hosts such as orange, guava, and mango.[76] Despite the specific attraction of male flies, they never ingest the chemical during exposure to **48**, unlike the case of *B. dorsalis* males in which they incorporate **44** as a pheromone precursor. In a laboratory test, lek-like behavior of *C. capitata* has been induced by either citrus leaves containing **48** or artificially by using a plastic leaf model thinly coated with **48**.[77] Mating occurred exclusively on the artificial leaves treated with **48**, suggesting the compound potentially serves as a chemical cue to facilitate orientation of flies to the rendezvous site. This represents an “external use” of plant volatiles for courtship other than (or in addition to) their own sex/aggregation pheromones.

In the white-spotted longicorn beetle, *Anoplophora malasiaca* (Cerambycidae), Yasui et al. (2007) [78] demonstrated that host plant sesquiterpenes (e.g. **47** in *Citrus* leaves) serve as a cue in their intraspecific communication. The host-shift of *Rhagoletis pomonella* (Tephritidae) from its native host downy hawthorn, *Crataegus mollis*, to introduced domesticated apple, *Malus domestica*, has been shown to be initiated by specific blends of their native host fruit volatiles where they rendezvous.[79]

Divergence of incipient populations and speciation in phytophagous insects may be driven by such host-related chemical cues, both in habitat-specific mating choice and in host preference by ovipositing females, although this warrants further in-depth investigations.

V. Floral volatiles in mutualistic associations

Pollination syndromes between higher plants and insects have resulted in the rich flora and fauna of the earth that we see today. Orchids are among the largest and most diverse group of the flowering plants, having radiated via highly specialized associations with specific pollinators. Mutualistic interactions between a group of orchids and pollinator fruit flies via floral synomones were examined as a collaboration projects with Dr. Keng-Hong Tan in a chemoecological study of pest fruit flies in the genus of *Bactrocera* (Tephritidae), particularly for species of economic importance.

Flowers of a fruit fly orchid, *Bulbophyllum cheiri* (Orchidaceae), attract males of several *Bactrocera* fruit fly species by floral methyl eugenol (**44**) in the rain forest of Southeast Asia.[80] The flower has a specialized hinged see-saw lip structure which temporarily traps the fruit fly between its lip and column, and transfers pollinia to the back of his thorax. The fly is rewarded by the floral attractant to be used as a precursor for defensive substances and sex pheromonal components (**45** and **46**) as described in Sections **III**-2 and **IV**-1, respectively. In this orchid-fruit fly association, both organisms gain direct reproductive benefits – the orchid flower gets pollinated without having to offer nectar, while the fruit fly boosts its pheromone and defense system with the attractant chemical **44**. Thus, the floral volatile is defined as synomone, an interspecific semiochemical that is adaptively advantageous to both the emitting and the receiving organisms. Flowers of another related orchid species, *Bulbophyllum vinaceum*, produce a complex mixture of phenylpropanoids including **45** and **46** (major), in addition to **44**, and attract ME-sensitive fruit flies (Fig. 5). The orchid flower seems to endow the male flies not only with precursor attractant (**44**) but also intact pheromone (**45** and **46**),

although the complete pollinator spectrum and actual role of such a multi-component system of attractant volatiles is unclear.[81)]

Another fruit fly orchid, *Bulbophyllum apertum* releases raspberry ketone (RK, **49**) in its fragrance to attract several RK-sensitive *Bactrocera* species such as the melon fly, *B. cucubitae*.[82)] The pollination takes place when a fly is compulsively feeding on the see-saw lip and momentarily trapped between the lip and column. The attractant chemical can act either as floral synomone (pollinarium-transported, as in *B. albistrigata*) or kairomone (accidental removal of pollinarium leading to total pollen wastage, as in *B. cucurbitae*) depending on the size of the visiting fruit fly species.

Flowers of *Bulbophyllum patens* attract male of a variety of *Bactrocera* fruit fly species, which show affinity to either **44** or **49**. The attractant component was identified as zingerone (**50**), a pungent essence of ginger.[83)] Zingerone conforms to a chemical structure resembling both **44** and **49**, and thus shows a potency to attract both ME-sensitive and RK-sensitive fruit fly species (e.g. *B. dorsalis*, *B. carambolae*, *B. umbrosa*, *B. cucurbitae* and *B. caudata*), thereby securing effective pollinators in the complex and dense vegetation in the rain forests. It was demonstrated that *B. cucurbitae* males that have fed on **50** sequester the intact compound in the rectal pheromone glands[83)] and become more successful in mating than unfed males.[84)] Interestingly, males of an Australian fruit fly, *B. jarvisi* (a pest of mango fruit) whose specific attractant was initially unknown, was found to be selectively attracted to **50** in the flowers of *Bulbophyllum baileyi* that the male flies frequently visit.[85)] This exemplifies a possible use of floral synomones as new monitoring agents for several specific pest species in the orchards.[86)]

VI. Conclusion

Insects effectively recognize host plants that produce specific primary and secondary metabolites via their finely tuned chemoreceptors. In a similar process, insects reject non-hosts or harmful plants. Host adaptation and speciation in insects are

tightly connected to such chemosensory mechanisms – interactions between specific ligands and chemoreceptors. One of the gustatory receptors involved in host recognition for the citrus swallowtail butterfly, *P. xuthus*, specific to synephrine (**8**) (Fig. 1) has recently been characterized from female tarsal chemosensilla.[87] A female-specific chemoreceptive protein was shown to specifically bind to a specific oviposition stimulant, aristolochic acid I (**17**) for the pipevine swallowtail butterfly, *A. alcinous*.[88] Numerous molecular techniques related to biosynthesis (in plants), biotransformation or detoxification (in insects) have been employed to provide evidence for possible (co)evolutionary processes between insects and plants.[89] The identification of genes related to these processes certainly opens the door to understanding the evolution of host specificity in herbivorous insects. The use of phytochemical cues by insects, both in host finding and in sexual communication, may facilitate a host shift and drive the process of colonization to a new adaptive zone with an explicit genetic isolation from the old host and community. The phytochemical mediation of sexual communication, including rendezvous cues (**IV**-2) in conjunction with host recognition (**I**-1, **I**-2), may be of great importance particularly at the initial stage of speciation, although such a process has not been well understood.[75,79]

On another front, phytochemical cues and their derivatives also provide us with innovative tools for pest management. However, in the case of fruit fly attractants such as the synomones mediating orchid-fruit fly interactions, the intensive use of such allelochemicals in agricultural fields may threaten their comunications and their populations, in the natural habitat. We need to understand more about chemical ecological networks in natural ecosystems as well as agricultural ecosystems, to seek a better way to live together.

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Figure Legends

Fig. 1. Plant metabolites as host-finding cues in butterflies and aphids.
Oviposition stimulants of *Papilio xuthus*: hesperidin (**1**), narirutin (**2**), rutin (**3**), vicenin-2 (**4**), adenosine (**5**), 5-hydroxy-*N*ω-methyltryptamine (**6**), bufotenine (**7**), (–)-synephrine (**8**), (+)-*chiro*-inositol (**9**), (–)-stachydrine (**10**); *P. macilentus*: cnidioside A (**11**); *P. bianor*: (–)-2*C*-methyl-D-erythrono-1,4-lactone (**12**), (–)-4-(*E*)-caffeoyl-L-threonic acid (**13**); *P. polyxenes*: luteolin 7-*O*-(6”-*O*-malonyl)-β-D-glucopyranoside (**14**), tyramine (**15**), chlorogenic acid (**16**); *Atrophaneura alcinous*: aristolochic acid I (**17**), sequoyitol (**18**), 3-hydroxy-4-methoxycinnamoylmalic acid (**19**); *Battus philenor*: **17** and pinitol (**20**); *Luehdorfia japonica*: isorhamnetin 3-*O*-glucopyranosyl-(l→6)-galactopyranoside-7-*O*-glucopyranoside (**21**). Larval feeding stimulant of *P. xuthus*: citrusin I (**22**), isosinensetin (**23**), 1,2-dilinolenoyl-3-galactopyranosyl-sn-glycerol (**24**). Probing stimulants in an aphid, *Megoura crassicauda*: quercetin 3-*O*-α-L-arabinopyranosyl-(1→6)-(2”-*O*-(*E*)-*p*-coumaroyl)-β-D-galactopyranoside (**25**).

Fig. 2. Plant metabolites as chemical barrier against insects.
Oviposition and feeding deterrents of *P. xuthus*: quercetin 3-*O*-(2^{G}-β-D-xylopyranosylrutinoside) (**26**), 5-{[2-*O*-(β-D-apiofuranosyl)-β-D-glucopyranosyl]oxy}-2-hydroxybenzoic acid (**27**) and disyringoyl glucaric acid (**28**). *Probing deterrent in aphids*, *Megoura crassicauda*: (*E*)-2-methyl-2-butene-1,4-diol 4-*O*-β-D-glucopyranoside (**29**). Insect growth regulators: juvenile hormone III (**30**), juvocimene II (**31**), 1-(3,4-methylenedioxyphenyl-(*E*)-3-decene (juvadecene) (**32**).

Fig. 3. Defensive substances sequestered from plants.
Idea leuconoe: ideamine B (**33**); *Parnassius apollo* and *Abraxas glossulariata*:

sarmentosin (**34**); *Arichanna gaschkevitchii*: arichannatoxins I (**35**); *Acyrthosiphon nipponicus*: paederoside (**36**); *Athalia rosae ruficornis*: clerodendrin D (**37**); *Diabrotica speciosa* and *Cerotoma arcuata*: 23,24-dihydrocucurbitacin D (**38**).

Fig. 4. Plant chemicals for sexual communication.

Males of the giant danaine butterfly, *Idea leuconoe*, biotransform defensive pyrrolizidine alkaloid, lycopsamine (**39**), to danaidone (**40**) and viridifloric β-1actone (**41**) and emit from the hairpencil organ as sex pheromone. A female oriental fruit moth, *Grapholita molesta*, is attracted to male sex pheromone composed of methyl epijasmonate (**42**) and ethyl (*E*)-cinnamate (**43**). Males of oriental fruit fly, *Bactrocera dorsalis*, pharmacophagously acquire methyl eugenol (**44**) from plants and biotransform to sex pheromone 2-allyl-4,5-dimethoxyphenol (**45**) and (*E*)-coniferyl alcohol (**46**), which entice females during courtship. Males of the guava fruit fly, *B. correcta*, sequester β-caryophyllene (**47**) in the rectal pheromone glands. Mediterranean fruit fly, *Ceratitits capitata*, may use α-copaene (**48**) as a cue to navigate both sexes to the rendezvous site.

Fig. 5. Floral synomone of fruit fly orchids attracting pollinator *Bactrocera* fruit flies.

Bulbophyllum vinaceum emits methyl eugenol (ME, **44**); *Bulbophyllum apertum* produces raspberry ketone (RK, **49**); *Bulbophyllum patens* emits zingerone (ZN, **50**) which has a hybrid structure between **44** and **49** and attracts both ME-sensitive (e.g. oriental fruit fly, *B. dorsalis*) and RK-sensitive species (e.g. melon fly, *B. cucurbitae*).

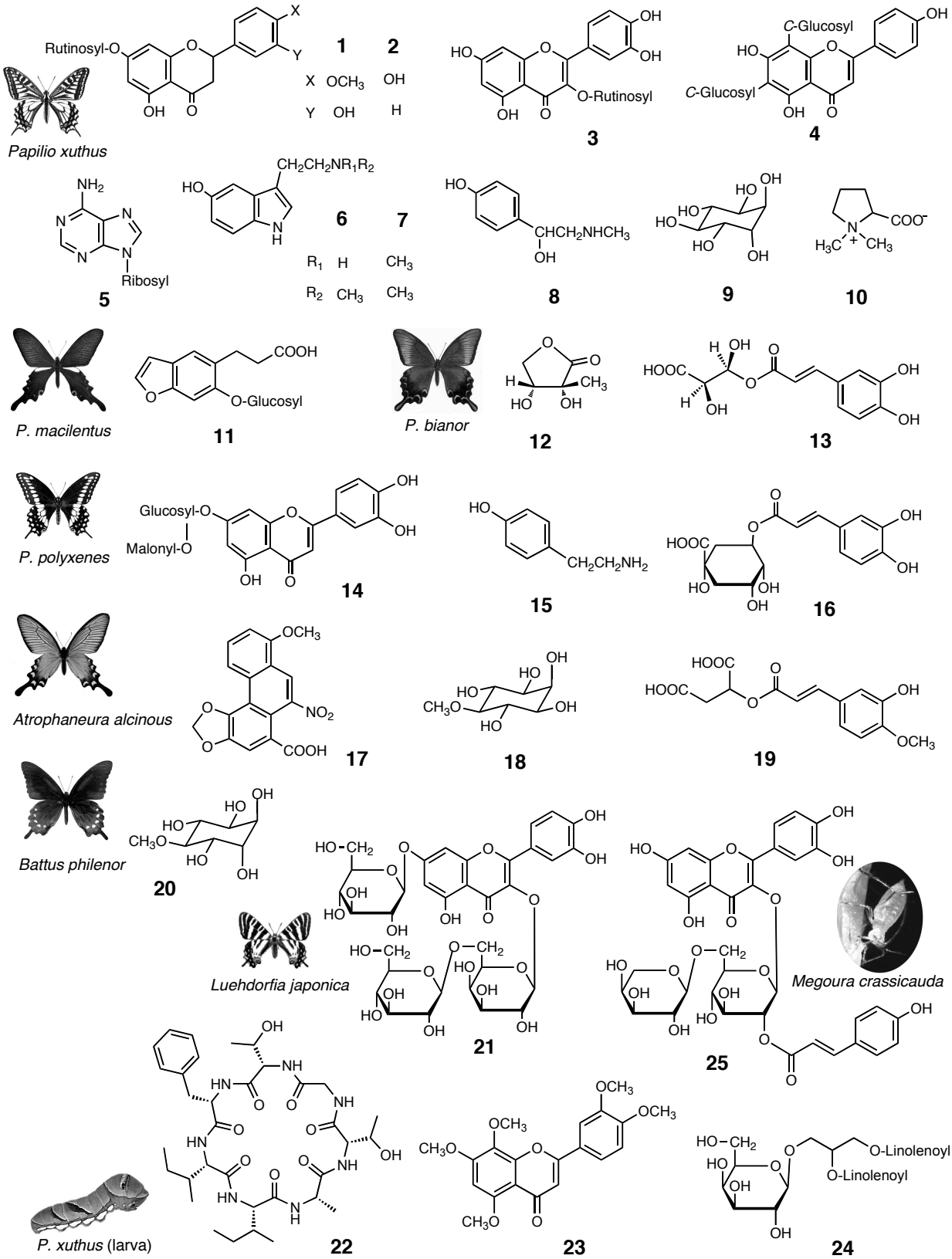


Fig. 1. Nishida

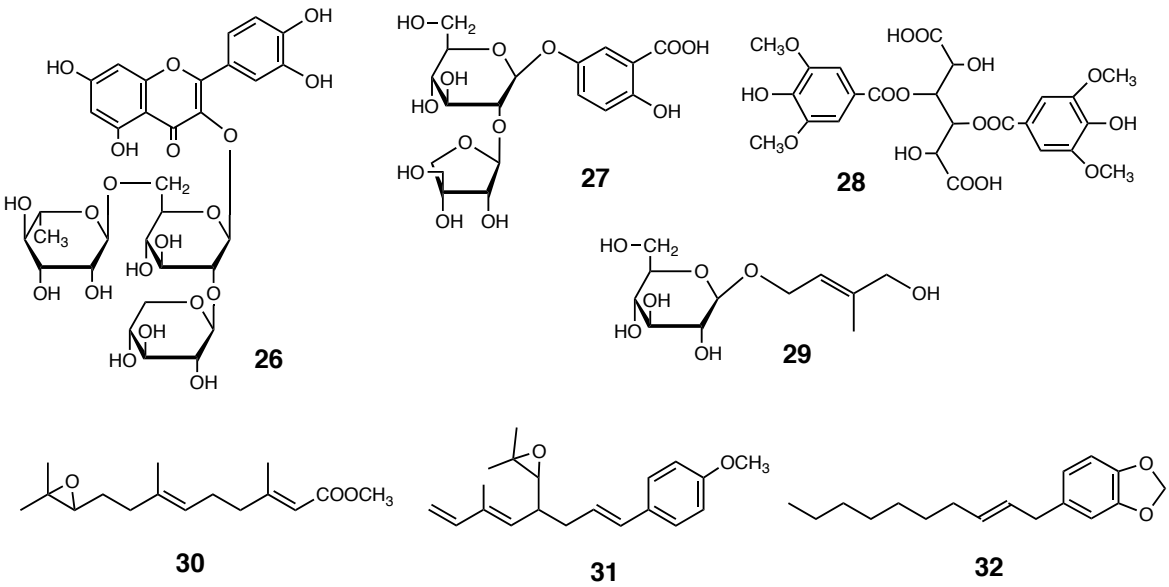
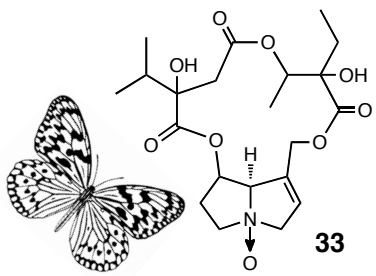


Fig. 2. Nishida



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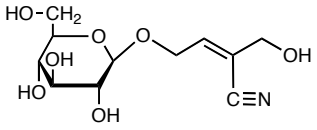
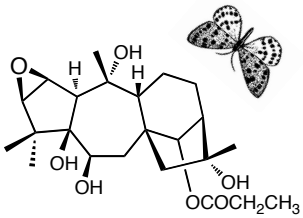
Idea leuconoe



34

Parnassius phebus

Abraxus miranda



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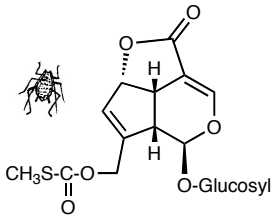
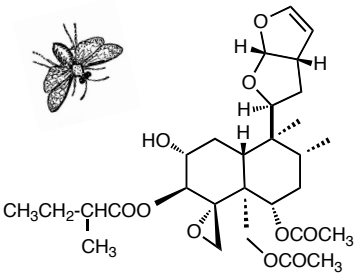
Arichanna gaschkevitchi



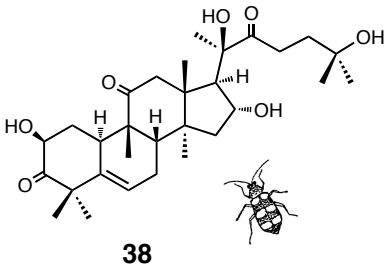
36

Acyrthosiphon nipponicus



Athalia rosae ruficornis

37



38

Diabrotica speciosa

Fig. 3. Nishida

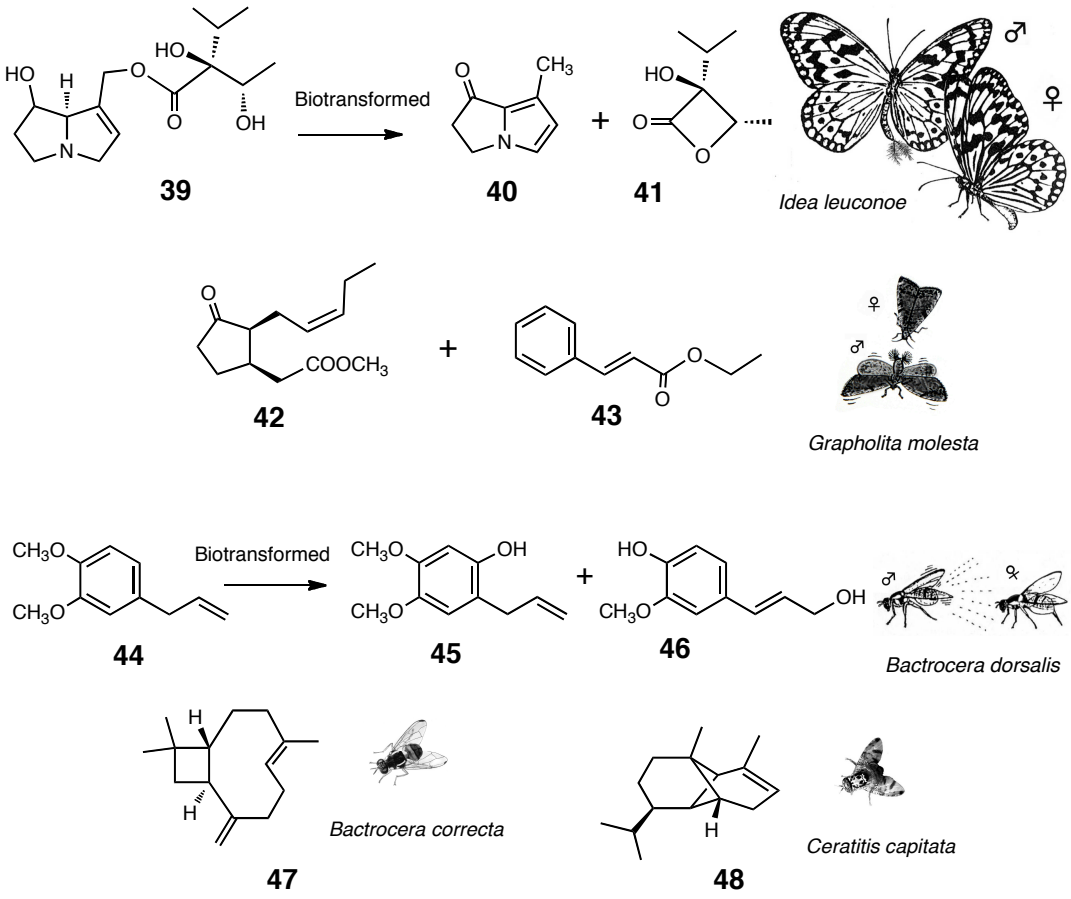


Fig. 4. Nishida

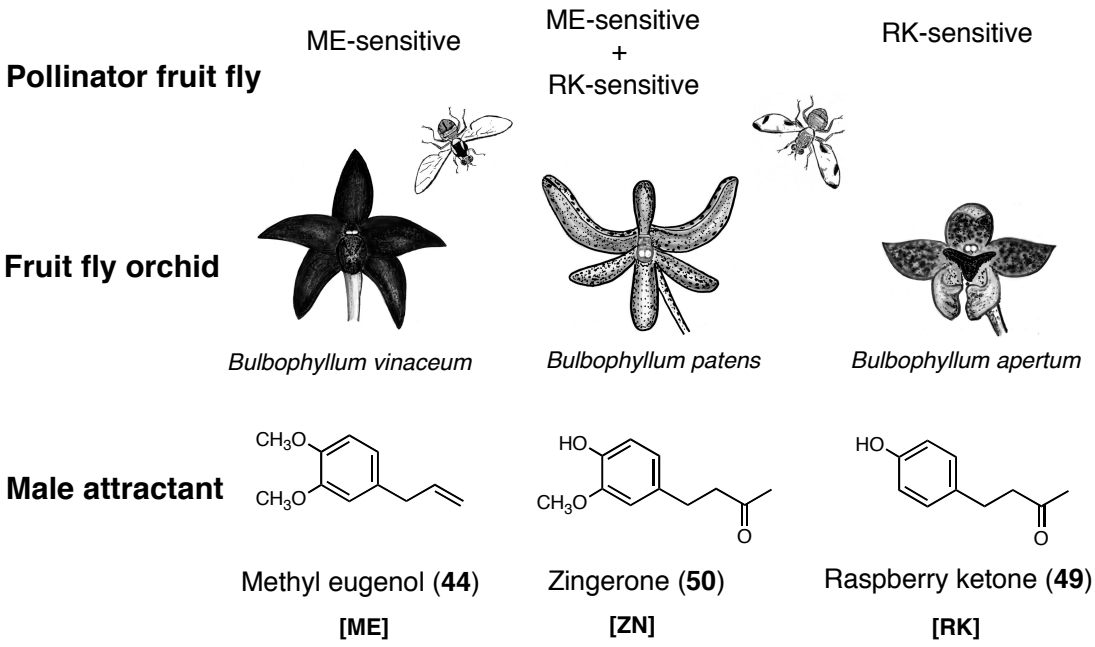


Fig. 5. Nishida